PREPARATION OF ANTISERUM AGAINST MYOSIN OF HUMAN SMOOTH MUSCLE

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A method of obtaining antiserum against myosin from the smooth muscle of the human uterus in rabbits immunized by injection of a precipitate consisting of myosin and the corresponding antibodies into the popliteal lymph glands is described. In sections from various organs from man and animals (mouse, rat) stained by the indirect Coons' method the antiserum revealed only smooth-muscle cells regardless of their localization.

Demonstration of the antigenic specificity of myosin from smooth and striated muscle [3, 10] has provided reliable evidence of the histogenesis and function of different types of cells by means of immunofluorescence tests. The differential diagnosis of human myogenic tumors has been simplified [4, 13, 14]. The participation of smooth-muscle cells in the formation of atherosclerotic plaques has been proved [15, 18]. A definite role has been ascribed to them in the contraction of healing wounds [11]. There is evidence of differences in the histogenesis of Aschoff nodules in the myocardium and endocardium [8]. Previous notions regarding the contractility of the myoepithelium of glands and cells of the mesangium of the renal glomeruli has been confirmed by detection of smooth-muscle myosin in their cytoplasm [5, 7].

In the investigations cited above most workers used immune sera against homologous and heterologous preparations of actomyosin and myosin from smooth muscles. For some purposes the sera of patients with hepatitis [9], rheumatic fever [1], and bronchial asthma [17], containing antibodies against unknown smooth-muscle antigens are evidently suitable also. Whatever the case, however, a strict immunological control, excluding crossed reactions, is essential. However, this has not satisfied the demands presented to it in all investigations [5, 15], partly on account of the difficulty of isolating purified myosin [2, 16]. This also explains attempts to obtain a more reliable and convenient method of obtaining monospecific antiserum against smooth-muscle myosin.

On this occasion myosin was extracted from the surgically removed uterus with 0.6 M KCl solution in 0.02 M phosphate buffer, pH 7.0, followed by reprecipitation four times with 12 volumes of distilled water [2]. About 10 ml of myosin solution containing 10-12 mg/ml protein by Lowry's method was obtained from 100 g myometrium. The sera of rabbits immunized with myosin in Freund's adjuvant, and later in solution (15 mg protein per rabbit), proved to be heterogeneous. After exhaustion with plasma all the antisera formed two or three bands in the agar diffusion test with various concentrations of myosin (1% agar and the myosin dilutions were made up in 0.6 M KCl solution in 0.02 M phosphate buffer, pH 7.0). In infections treated by the indirect Coon's method using pure ass antibodies against rabbit γ-globulin [6] the antisera caused fluorescence not only of the smooth muscles, but also of the connective-tissue structures of many organs. Absorption of the antisera by homogenates of various tissues (spleen, liver, dura mater, ligaments, hyaline cartilage) preliminarily extracted for 1-24 h with 0.6 M KCl solution to remove the myosin from the vessels, proved ineffective: antibodies against connective tissue still remained or the fluorescence of the smooth muscles was sharply reduced. The contaminating antibodies were removed by immunization with the individual precipitation arcs [12].

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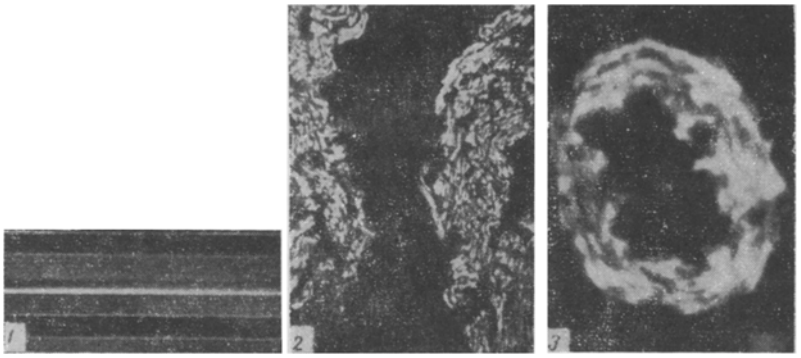

Fig. 1 Fig. 2 Fig. 3.

Fig. 1. Characteristics of original antiserum against smooth-muscle myosin preparation from human uterus. Two bands, principal and accessory, are formed in the agar diffusion test. Top gutter contains antiserum, bottom gutter myosin solution.

Fig. 2. Specific fluorescence of smooth muscles in middle third of human esophagus. Connective-tissue structures and fibers of striated muscles (in center) remain dark. Treated with antiserum of rabbit immunized with precipitate of smooth-muscle myosin with corresponding antibodies, by indirect Coon's method, 50×.

Fig. 3. Specific fluorescence of muscular coat of artery from the human liver. Treated with fewer antibodies against smooth-muscle myosin by the indirect Coons' method, 120×.

Parallel gutters 50 mm in length and 2 mm in width, cut out of the agar, were filled with myosin and antiserum against it in dilutions giving optimal separation of the precipitation bands (Fig. 1). The principal band, lying closer to the gutter with antigen and corresponding to myosin [10], was cut out with a razor together with the agar and washed for three days in buffered 0.6 M KCl solution. The agar was then crushed in a glass homogenizer and thoroughly mixed with an equal volume of Freund's complete adjuvant (Difco). Intact rabbits were injected each with 0.5 ml of the mixture directly into both popliteal lymph glands and the underlying cellular tissue. After one month had elapsed twice the dose of homogenized precipitate without the adjuvant was injected subcutaneously in the dorsal region. Blood was collected one week after the last injection. Concentrated sera of two of the three immunized rabbits formed a single band in the agar diffusion test with myosin.

Active antisera showed up only the muscular coat of the blood vessels and bronchi and the smooth muscles of the esophagus, intestine, stomach, and uterus in sections of organs from man and various animals (mouse, rat) (Fig. 2). Nevertheless, all the antisera were additionally absorbed by homogenate of human hyaline cartilage.

By using a technique of preparing the immunosorbent with glutaraldehyde [6] it was possible to elute fewer antibodies against myosin, which in a low concentration reduced the background fluorescence to a minimum (Fig. 3).

Although the method described lengthens the immunization procedure and makes it rather more complicated, it nevertheless gives a definite guarantee that a monospecific antiserum against smooth-muscle myosin will be obtained. It is evidently worthwhile using this method both to determine the precise distribution of myosin and also to investigate the localization of other specific muscle proteins.

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